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Glutathione Levels and Glutathione-Glutamate Correlation in Patients With Treatment-Resistant Schizophrenia

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Treatment-resistant schizophrenia (TRS) has been suggested to involve glutamatergic dysfunction. Glutathione (GSH), a dominant antioxidant, is known to be involved in glutamatergic neurotransmission. To date, no study has examined GSH levels in patients with TRS. The aim of this study was to examine GSH levels in the dorsal anterior cingulate cortex (dACC) of patients with TRS. Patients with schizophrenia were categorized into 3 groups with respect to their antipsychotic response: (1) clozapine (CLZ) nonresponders, (2) CLZ responders, and (3) first-line responders (FLR). GSH and glutamine + glutamate (Glx) levels were measured using 3T proton magnetic resonance spectroscopy. Firstly, dACC GSH levels were compared among the patient groups and healthy controls (HCs). Further, relationships between GSH and Glx levels were compared between the groups and GSH levels were explored stratifying the patient groups based on the glutamate-cysteine ligase catalytic (GCLC) subunit polymorphism. There was no difference in GSH levels between the groups. FLR showed a more negative relationship between GSH and Glx levels in the dACC compared to HCs. There were no effects of GCLC genotype on the GSH levels. However, CLZ responders had a higher ratio of high-risk GCLC genotype compared to CLZ nonresponders. This study demonstrated different relationships between GSH and Glx in the dACC between groups. In addition, the results suggest a potential link between CLZ response and GCLC genotype. However, it still remains unclear how these differences are related to the underlying pathophysiology of schizophrenia subtypes or the mechanisms of action of CLZ.

Key words: schizophrenia/oxidative stress/glutamate/glutathione/treatment-resistant

Introduction

Schizophrenia is a debilitating illness, which affects approximately 1% of the global population.[1] Most antipsychotics share the property of dopamine antagonism and play a central role in the pharmacological treatment of schizophrenia.[2] Based on the clinical effects of antipsychotics, the dopamine hypothesis of schizophrenia was proposed, positing that elevated dopaminergic function plays a central role the pathophysiology of schizophrenia.[3,4] In support, one meta-analysis of positron emission topography (PET) studies demonstrated that presynaptic dopamine function is higher in patients with schizophrenia compared to healthy controls (HCs).[5] Indeed, greater endogenous dopamine levels at D_2 receptors have been demonstrated in the dorsal striatum of patients with schizophrenia,[6,7] and these elevated dopamine levels positively predict response to antipsychotics.[8,9] However, it is also known that about one-third of patients with schizophrenia do not respond to non-clozapine (CLZ) antipsychotics; these patients are considered to have treatment-resistant schizophrenia (TRS). Notably, previous [^{18}F]-DOPA PET studies have

demonstrated lower dopamine synthesis capacity in the striatum of patients with TRS compared to those who respond to first-line antipsychotics (first-line responders: FLR), ie, antipsychotics other than CLZ,[10,11] suggesting that TRS represents a different underlying pathophysiology than dopamine dysfunction.

It has been proposed that the glutamate (Glu) hypothesis may play a role in the pathophysiology of TRS.[12,13] Studies noted elevations of Glu levels in the dorsal anterior cingulate cortex (dACC) of patients with TRS compared to FLR or HCs using proton magnetic resonance spectroscopy (^{1}H-MRS).[14–16] In addition, a recent prospective study demonstrated that higher Glu levels in the pregenual ACC (pgACC) before antipsychotic treatment were related to treatment failure after 4 weeks of amisulpride treatment in patients with first-episode psychosis.[17] Furthermore, our group previously reported that patients with schizophrenia, who were resistant to other antipsychotics as well as CLZ (CLZ nonresponders), had higher glutamine + glutamate (Glx) levels in the dACC compared to HCs.[18] These findings suggest that the pathophysiology of TRS may be related to glutamatergic rather than dopaminergic dysfunction.

Glutathione (GSH) is another neurometabolite that plays a role in the glutamatergic system. It is known that GSH is involved in glutamatergic neurotransmission by potentiating N-methyl D-aspartate (NMDA) receptor functioning through activating the redox modulatory site.[19] Further, it has been demonstrated that the GSH cycle molds the activity of synaptic Glu.[20] In addition to its effects on glutamatergic neurotransmission, GSH is known to be a dominant antioxidant compound in the brain.[21]

Notably, oxidative stress and antioxidant defense dysfunction have been proposed as one of the putative mechanisms underlying schizophrenia.[21,22] It has been reported that GSH levels are reduced in the blood and in postmortem brains within this population[23–25]; in addition, a genetic study suggested relationships between polymorphisms of glutamate-cysteine ligase catalytic subunit (GCLC), a subunit of GSH synthesis enzymes, and risk of schizophrenia.[26] That study reported that the high-risk GCLC genotype was related to lower GSH levels in the pgACC regardless of illness condition (schizophrenia or HCs). Moreover, they also found that GSH levels were positively correlated with Glu levels in the ACC in those with low risk, but not in those with high risk.[27] Further, a recent meta-analysis of ^{1}H-MRS studies revealed lower ACC GSH levels in patients with schizophrenia compared to HCs, with a modest effect size (ES = 0.26).[28] Interestingly, one study reported that patients with schizophrenia taking CLZ had higher plasma GSH levels than those taking risperidone.[29] Another study reported that the nonfunctional polymorphism of glutathione S-transferases was related to higher susceptibility to TRS.[30] These results suggest a potential link of GSH metabolism to the pathophysiology of TRS and the effects of CLZ. However, to date no study has examined ACC GSH levels, and their relationship with ACC Glu levels, in patients with TRS.

In this study, we sought to examine the relationships between antipsychotic response/resistance and GSH levels in the dACC of patients with schizophrenia. We chose the dACC as the region of interest as postmortem studies reporting altered GSH levels have focused on this region.[21] Further, we classified patients with schizophrenia into the following 3 groups based on their response to antipsychotics: (1) CLZ nonresponders, (2) CLZ responders, and (3) FLR. We compared GSH levels in the dACC among the patient groups and HCs. We also examined relationships between dACC GSH levels and symptom severity. Furthermore, stratifying the patients based on the GCLC polymorphism, dACC GSH levels and the associations between GSH and Glu levels in the dACC were explored in the whole patient sample.

Methods

Participants

This was a single-center cross-sectional study conducted at the Centre for Addiction and Mental Health (CAMH) between 2014 and 2017. All participants were recruited from the CAMH Research Registry, study advertisements, or referrals. Each participant provided written informed consent, was screened for drugs of abuse as part of the screening visit, and received a stipend for their involvement. The study was approved by the Research Ethics Board at CAMH. Patients with a DSM-IV/SCID diagnosis of schizophrenia spectrum disorders were recruited, and antipsychotic treatment resistance was defined by the modified Treatment Response and Resistance in Psychosis (TRRIP) Working Group Consensus criteria.[31]

CLZ nonresponder criteria included: (1) current treatment of CLZ, (2) a history of treatment failure to optimal treatment with at least 2 previous non-CLZ antipsychotics, and (3) subsequent treatment failure with CLZ after patients had taken CLZ for ≥6 weeks at a minimum dose of 300 mg/day. CLZ responder criteria included: (1) current treatment of CLZ, (2) a history of treatment failure to optimal treatment with at least 2 previous non-CLZ antipsychotics, and (3) subsequent treatment response to CLZ. FLR criteria included: (1) current treatment of a single non-CLZ antipsychotic, and (2) treatment response. Lastly, HCs met inclusion criteria if they had no history of psychiatric illness, as assessed by the Mini-International Neuropsychiatric Interview (MINI).[32] Exclusion criteria for all groups consisted of: (1) substance abuse or dependence within the past 6 months; (2) positive urine drug screen at inclusion or prior to magnetic resonance imaging (MRI) scanning; (3) history of head trauma resulting in loss of consciousness >30 minutes; (4) an unstable physical

illness or neurological disorder; or (5) current administration of lamotrigine, topiramate, or memantine. The definition of optimal antipsychotic treatment and the assessment procedures of antipsychotic treatment response and failure were detailed elsewhere.[18]

MRI Acquisition

All participants were scanned in a 3T 750 MR scanner (General Electric HealthCare) with an 8-channel receive only head coil for reception and body coil for transmission at CAMH. For MRS voxel placement and gray-white matter segmentation, a 3-dimensional IR-prepared T1-weighted MRI scan (BRAVO, TE = 3.00 ms, TR = 6.74 ms, TI = 650 ms, flip angle = 8°, FOV = 230 mm, 256 × 256 matrix, slice thickness = 0.9 mm) was performed

^{1}H-MRS Acquisition

GSH spectra were obtained using the interleaved J-difference editing method (MEGA-PRESS, TE = 68 ms, TR = 1500 ms, spectral width = 5000 Hz, 4096 data points, 512 water-suppressed, 16 water-unsuppressed averages, 8 NEX), as previously described.[33–35] A voxel with the size of 24 ml (20 × 40 × 30 mm^3) was placed over the dACC (supplementary figure 1). Shimming was performed using the manufacture automated shimming routine (AUTOSHIM), to achieve a full-width at half maximum (FWHM) ≤ 10 Hz. Two frequency selective radio frequency pulses, with a pulse width of 14.4 ms, were used to invert the strongly coupled resonances of α (4.56 ppm) and β (2.95 ppm) protons of the cysteinyl moiety of GSH. The frequencies of the editing pulses alternated between editing "on" and editing "off" which were centered at 4.56 and 7.50 ppm, respectively. Raw MRS data from each coil were combined in the time domain based on coil sensitivity[36] from the unsuppressed water signal, weighted by the sum of squares of the signal intensities from each coil. Upon subtraction of the "on" and "off" conditions, the uncontaminated GSH resonance at 2.95 ppm is observed. IDL-based software (XsOs-NMR) was used to quantify the GSH and unsuppressed water peaks.[37] The data were spectrally apodized with a 3-Hz Gaussian filter and then zero-filled to 8192 points, prior to being Fourier-transformed. GSH resonances at 2.95 ppm were modeled as a singlet using pseudo-voigt fitting functions and then fitted in the frequency domain using a highly optimized public-domain Levenberg-Marquardt nonlinear least-squares minimization routine, MPFIT (supplementary figure 2).[38] In this study, the ratio of GSH to unsuppressed water peak (GSH/H_2O) areas is reported in institutional units (IU). Spectra quality was visually assessed by 2 authors (P.T. and N.S.).

Glu and Glx spectra were collected using point-resolved spectroscopy (PRESS, TE = 35 ms, TR = 2000 ms, spectral width = 5000 Hz, 4096 data points, 128 water-suppressed, 16 water-unsuppressed averages, 8 NEX). Shimming was performed to achieve a full-width at FWHM ≤ 12 Hz, measured on the unsuppressed water signal from the voxel. ^{1}H-MRS voxels were placed on the bilateral dACC (voxel size = 9.0 ml [30 × 20 × 15 mm^3]) (figure 1). The detailed voxel placement procedures, locations of the ^{1}H-MRS voxels, and representative spectra were provided

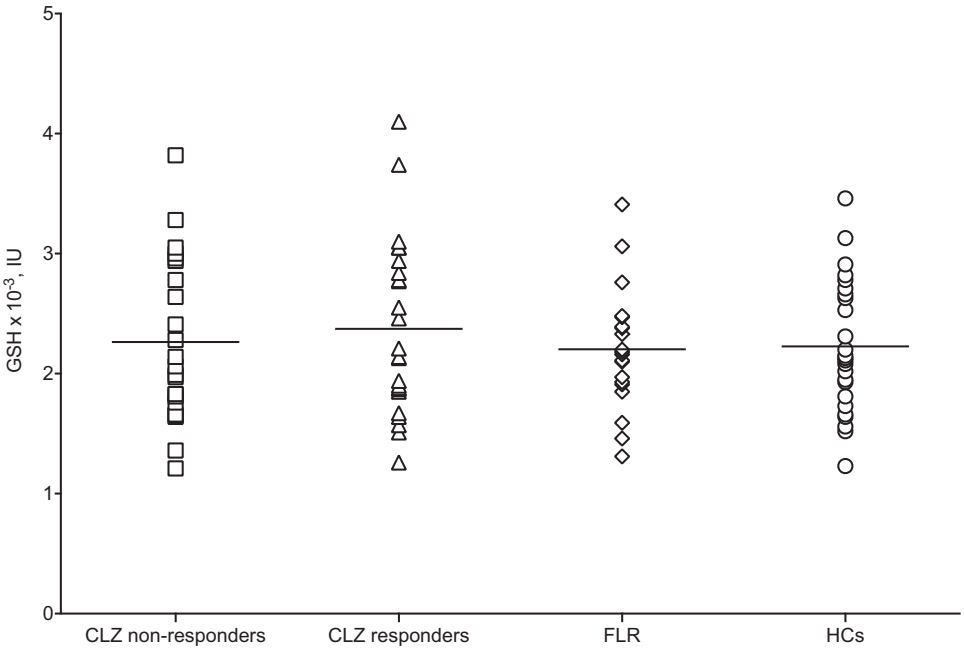

Fig. 1. GSH levels in the dACC. *Note*: CLZ, clozapine; dACC, dorsal anterior cingulate cortex; FLR, first-line responders; GSH, glutathione; HC, healthy control; IU, institutional units.

elsewhere.[18] Water-suppressed spectra were analyzed using LCModel version 6.3-0E.[39] Glu levels were estimated with a field appropriate LCModel-provided basis set with matching TE (=35 ms). Then, metabolite levels were normalized to unsuppressed water signal. Metabolite levels were expressed as IU. %SD values ≥20% were deemed poor quality and excluded from subsequent analyses.

T1-weighted MRI scans were segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) using the FIRST tool from FSL.[40] A MATLAB-based software package named "Gannet" (http://www.gabamrs.com) was used to create a mask of the voxel size and location on the segmented T1-weighted image, permitting correction of neurometabolite level for fractions of CSF in the region of interest (ROI),[41] corrected levels = water reference levels * (100[Total volume]/100 − CSF%[=GM% + WM%]).

Genotyping of GCLC Trinucleotide Polymorphism

Classification into high-risk or low-risk genotype was based on the number of GAG repeats as described by Gysin et al (7/8, 8/8, 8/9, and 9/9 and 7/7 and 7/9, respectively).[26] Detailed genotyping procedures are provided in the supplementary methods.

Statistical Analysis

Statistical analyses were performed using SPSS Statistics version 25 (IBM Corporation). Firstly, clinical and demographic characteristics, FWHM values, and GM ratios (GM/[GM + WM]) within GSH voxel were compared between the groups. Then, relationships between GSH levels, clinical and demographic characteristics, and GM ratios were examined within each group. A significance level of $P < .05$/number of comparisons was utilized in each group analyses.

For primary analyses, group differences in dACC GSH levels were examined. Firstly, GSH levels were compared between the groups using an analysis of variance (ANOVA). Then, analyses of covariance were performed controlling for age, GM ratio, FWHM, and characteristics that showed associations with GSH levels. For exploratory analyses, correlations between GSH levels and symptom severity scores were examined with using a significance threshold of $P < .0125$ (0.05/4), owing to the number of comparisons (Positive and Negative Syndromes Scale [PANSS] total and 3 subscale scores) in the whole patient sample and within each group by using Spearman's correlation. In addition, we assessed relationships between GSH and glutamatergic neurometabolite levels within each group with using a significance threshold of $P < .0125$ (0.05/4). Further, differences in associations between dACC GSH and glutamatergic neurometabolite levels among groups were examined by using Fisher's *r*-to-*z* calculation with using a significance threshold of $P < .0083$ ($0.05/6[={}_4C_2]$), owing to the 4 group comparisons.

As the genetic samples were collected only for the patient groups, a 2-way ANOVA was conducted to examine effects of the GCLC genotype (high-risk or low-risk) and group of patients (CLZ nonresponders, CLZ responders, or FLR) on GSH levels only for the patient samples. When there were any statistically significant genotype-by-group interactions ($P < .05$), post hoc analyses were performed with ANOVA or chi-square test, adjusting significant *P* values by number of comparisons ($P < .016$ [3 patient group]).

Finally, the effects of GCLC genotype (high-risk or low-risk) on dACC GSH levels and on the associations between GSH and glutamatergic neurometabolite levels in the dACC were examined by 2-tailed *t* test in the whole patient sample.

Results

Clinical and Demographic Characteristics of Participants

A total of 98 participants were included in this study, which consisted of 24 CLZ nonresponders, 27 CLZ responders, 21 FLR, and 26 HCs. All participants were enrolled from our previous study that examined the relationship between glutamatergic neurometabolite levels and treatment response to antipsychotics including CLZ in patients with schizophrenia.[18] Clinical and demographic characteristics of the participants are presented in table 1. HCs showed a lower ratio of tobacco use compared to the patient groups. Chlorpromazine (CPZ) daily doses were higher in CLZ nonresponders compared to FLR. CLZ nonresponders showed higher symptom severity scores compared to CLZ responders and FLR. Six GSH data points (1 patient with CLZ nonresponder, 3 CLZ responders, 1 FLR, and 1 HC) were excluded from the analysis due to poor data quality. Eight participants did not agree to provide blood samples for genotyping (4 CLZ nonresponders, 2 CLZ responders, and 2 FLR).

GSH Levels and Spectrum Quality Indices

FWHM values and GM ratio were not different between the groups (table 2). The relationships between participants' clinical and demographic characteristics, GM ratios, and GSH levels are displayed in supplementary table 1. GM ratios were associated with dACC GSH levels in HCs while no other correlations were found. GSH levels in the dACC were not different among the groups (figure 1). The result did not change after controlling for age, GM ratio, or FWHM (table 2). Further, the results did not change after controlling for tobacco use for patient

Table 1. Characteristics of Participants

	CLZ Nonresponders (n = 24)	CLZ Responders (n = 27)	FLR (n = 21)	HCs (n = 26)	ANOVA or Chi-Square	
	Mean ± SD or n (%)	Mean ± SD or n (%)	Mean ± SD or n (%)	Mean ± SD or n (%)	F Value or df	P Value
Age, year	44.8 ± 13.2	40.5 ± 11.2	46.3 ± 12.7	40.8 ± 13.2	F(3,94) = 0.87	.46
Female	5 (20.8)	8 (29.6)	5 (23.8)	7 (26.9)	3	.90
Tobacco use	10 (41.7)	12 (44.4)	13 (61.9)	1 (3.8)	3	.0003[a]
DUI, year	23.5 ± 13.2	16.4 ± 9.7	20.0 ± 12.2		F(2,69) = 2.53	.09
CPZ equivalent dose, mg/day*	643.7 ± 186.5	527.1 ± 201.7	443.1 ± 188.1		F(2,69) = 6.18	.003[b]
CLZ dose, mg/day	429.1 ± 124.3	351.4 ± 134.5			F(1,50) = 4.56	.04
PANSS total score	82.7 ± 12.0	56.1 ± 10.9	57.2 ± 9.5		F(2,69) = 45.91	<.0001[c]
Positive subscale	22.5 ± 4.0	11.5 ± 2.0	10.9 ± 2.3		F(2,69) = 122.35	<.0001[c]
Negative subscale	20.6 ± 4.3	16.1 ± 4.8	16.0 ± 3.6		F(2,69) = 8.90	<.0001[d]
General subscale	39.6 ± 7.2	28.5 ± 5.6	30.3 ± 4.7		F(2,69) = 24.70	<.0001[c]

Note: Significant P values were set as <.005 (0.05/10). ANOVA, analyses of variance; CGI-S, Clinical Global Impression Severity scale; CLZ, clozapine; CPZ, chlorpromazine; DUI, duration of illness; FLR, first-line responders; HCs, healthy controls; LAI, long-acting injection; PANSS, Positive and Negative Syndromes Scale; SD, standard deviation.
*Antipsychotics: first-line responders were on flupenthixol (n = 1), haloperidol (n = 2), loxapine (n = 1), olanzapine (n = 8), paliperidone (n = 1), risperidone (n = 1), ziprasidone (n = 1), flupenthixol LAI (n = 2), fluphenazine LAI (n = 1), paliperidone LAI (n = 1), or risperidone LAI (n = 2).
Followings were Bonferroni-corrected P values < .05.
[a]CLZ nonresponders > HCs (P = .01), CLZ responders > HC (P = .005), FLR > HCs (P < .001).
[b]CLZ nonresponders > FLR (P = .003).
[c]CLZ nonresponders > CLZ responders (P < .001), CLZ nonresponders > FLR (P < .001).
[d]CLZ nonresponders > CLZ responders (P = .001), CLZ nonresponders > FLR (P = .002).

groups to consider its effects on antipsychotic levels, including CLZ. The results of the other neurometabolite levels were presented in the supplementary table 4.

Relationships Between GSH Levels and Psychopathological Scales

GSH levels in the dACC were not related to any symptom severity scores either within each group or the whole patient sample (supplementary table 2). The results remained unchanged after controlling for age, sex, tobacco use, and CPZ daily dose.

Correlations Between GSH and Glu in the dACC

CLZ nonresponders and HCs showed positive correlation between Glu and GSH levels in the dACC (figure 2). Regarding the differences in the correlation of Glu × GSH between the groups by the r-to-z calculation, FLR showed a more negative correlation compared to CLZ nonresponders (corrected-P = .006) and to HCs (corrected-P = .004). Regarding the correlations between Glx and GSH, not a significant correlation was observed in any group. FLR had a more negative correlation compared to HCs (corrected-P = .04). When partial correlation analyses were applied controlling for duration of illness, the difference between FLR and CLZ nonresponders in the GSH × Glx correlation became significant (corrected-P = .01). The group combined figures were displayed in the supplementary figure 3. Correlations between GSH and the other neurometabolite levels were presented in the supplementary figure 4. No significant between-group differences were observed in the other neurometabolites in the correlations to GSH.

GCLC Genotype in Patients

A group difference was found in proportion of high-risk or low-risk GCLC genotypes between the patient groups (P = .039). Post hoc analyses found a higher ratio of the high-risk genotype in CLZ responders compared to CLZ nonresponders (odds ratio = 5.25, corrected-P = .042) (table 3).

Effects of GCLC Genotype on GSH Levels and Correlations Between GSH and Glu Levels

There was no difference in GSH levels between patients with high-risk GCLC genotype and patients with low-risk GCLC genotype. The results did not change when only Caucasian patients were included in the analysis (supplementary table 3). Associations between GSH and Glu (and Glx) levels were not different between the groups (supplementary figure 5).

Discussion

This study examined the relationships between dACC GSH levels and treatment response to antipsychotics,

Table 2. GSH Levels in the dACC and Scan Quality Indices Between Groups

	CLZ Nonresponders (n = 23)	CLZ Responders (n = 24)	FLR (n = 20)	HCs (n = 25)	ANOVA		ANCOVA With Age Covariate		ANCOVA With GM/(GM + WM) Covariate	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	F Value	P Value	F Value	P Value	F value	P Value
GSH × 10^{-3}, IU	2.26 ± 0.67	2.37 ± 0.72	2.20 ± 0.50	2.22 ± 0.57	$F(3,88) = 0.34$	.80	$F(3,87) = 0.46$	.71	$F(3,87) = 0.46$	.71
GM/(GM + WM)	0.70 ± 0.06	0.70 ± 0.03	0.71 ± 0.03	0.69 ± 0.04	$F(3,88) = 0.63$	.60				
FWHM	7.04 ± 1.15	7.13 ± 1.15	7.45 ± 1.00	7.36 ± 1.15	$F(3,88) = 0.61$	.61				

Note: ANOVA, analysis of variance; CLZ, clozapine; dACC, dorsal anterior cingulate cortex; FLR, first-line responders; FWHM, full-width at half maximum; GM, gray matter; GSH, glutathione; HCs, healthy controls; IU, institutional units; SD, standard deviation; WM, white matter.

including CLZ, in patients with schizophrenia. We did not find any differences in dACC GSH levels among CLZ nonresponders, CLZ responders, FLR, and HCs. Further, GSH levels were not related to symptom severity in any of the groups. However, this study revealed different relationships between GSH and Glu (and Glx) levels in the dACC between groups; FLR showed a more negative relationship compared to HCs. In addition, a higher proportion of individuals with high-risk GCLC genotype were observed in CLZ responders compared to CLZ nonresponders. However, the previously reported effects of GCLC genotype on ACC GSH levels were not observed in this study.

A recent meta-analysis focusing on ^{1}H-MRS studies reported lower GSH levels in patients with schizophrenia compared to HCs, with a small effect size (ES = 0.26).[28] Of the included 12 studies in this meta-analysis, 10 did not find any differences in ACC GSH levels between patients with schizophrenia and HCs, which is consistent with our results. On the other hand, 2 studies have reported lower GSH levels in patients with schizophrenia compared to HCs.[42,43] Regarding their ROIs, both studies placed their ROIs on the rostral area of the ACC, while the current study examined the dACC. It should be noted that among the studies assessing Glu levels in patients with schizophrenia, alterations may be more apparent in the rostral, compared to dorsal, area of the ACC.[18] In addition, these 2 studies included individuals with relatively younger participants with a short duration of illness. In the study by Do et al, two-thirds of the patients had an illness duration that was shorter than 3 years (mean age of the patients was not reported).[42] The mean age and duration of illness of patients were 27.2 and 4.5 years, respectively, in the study by Kumar et al.[43] On the other hand, the mean age and the duration of illness of our study was 43.6 and 20.0 years, respectively. Although the aforementioned meta-analysis noted no significant association between ACC GSH levels and age or duration of illness,[28] aging and illness chronicity may account for our null finding related to dACC GSH levels. Furthermore, Kumar et al found that the effect size of lower GSH levels was larger in those with residual schizophrenia than those with nonresidual schizophrenia, and that the former group was largely responsible for their finding of lower ACC GSH levels compared with HCs. They included patients with a score ≥2 on the Signs and Symptoms of Psychotic Illness (SSPI) global negative scale (range 1–4) in the residual group. Comparatively, our subjects in FLR and CLZ responder groups showed less severe negative symptoms; average PANSS negative scores were 16 out of 49. Therefore, the null finding of this study could partly be attributed to the location of ACC ROI, age, and duration of illness of the included patients, and the lack of residual type in our sample.

We did not find any relationships between GSH levels and symptom severity measures. To our knowledge,

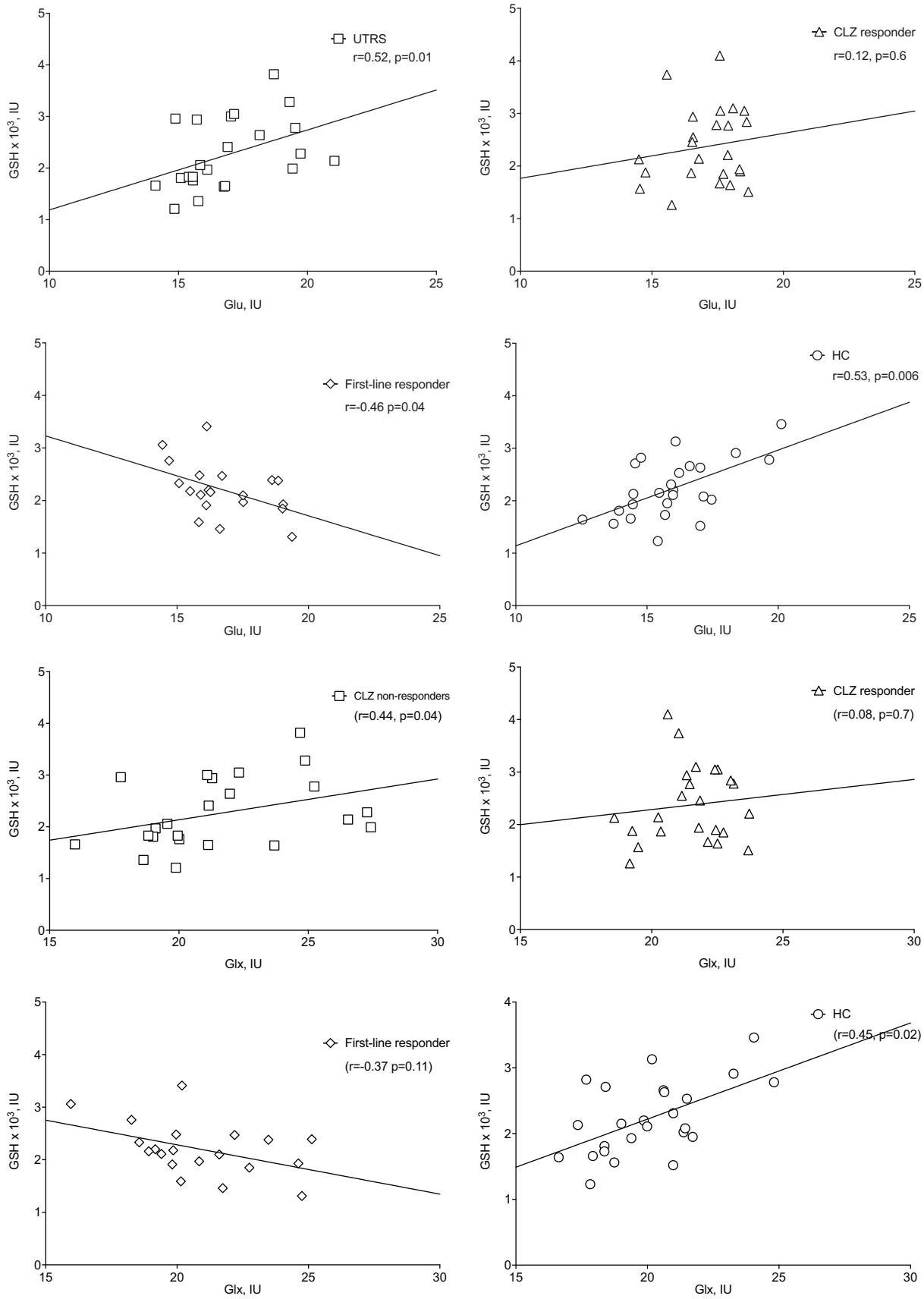

Fig. 2. Relationships between GSH and Glu and Glx in each group *Note*: CLZ, clozapine; dACC, dorsal anterior cingulate cortex; FLR, first-line responders; Glu, glutamate; Glx, glutamate + glutamine; GSH, glutathione; HC, healthy control; IU, institutional units.

Table 3. GCLC GAG TNR Genotypes and Ethnicity in Patient Groups

	CLZ Nonresponders	CLZ Responders	FLR	Chi-Square	
	(%)	*n* (%)	*n* (%)	df	*P* Value
Genotypes	*n* = 19	*n* = 25	*n* = 19	2	.041[a]
High-risk (7/8, 8/8, 8, 8/9, 9/9)	10 (50.0)	21 (84.0)	11 (57.9)		
Low-risk (7/7, 7/9)	10 (50.0)	4 (16.0)	8 (42.1)		
Genotypes (only with Caucasian)	*n* = 13	*n* = 21	*n* = 17	2	.052
High-risk (7/8, 8/8, 8, 8/9, 9/9)	8 (61.5)	19 (90.5)	10 (58.8)		
Low-risk (7/7, 7/9)	5 (38.5)	2 (9.5)	7 (41.2)		
Ethnicity	*n* = 20	*n* = 25	*n* = 19	8	.16
Caucasian	13 (65.0)	21 (84.0)	17 (89.4)		
African descent	1 (5.0)	1 (4.0)	1 (5.3)		
East/southeast Asian	0	2 (8.0)	0		
Hispanic	2 (10.0)	0	0		
Other	4 (20.0)	1 (4.0)	1 (5.3)		
Ethnicity	*n* = 20	*n* = 25	*n* = 19	3	.44
Caucasian	13 (65.0)	21 (84.0)	17 (89.4)		
Non-Caucasian	7 (35.0)	4 (16.0)	2 (10.6)		

Note: CLZ, clozapine; FLR, first-line responders; GCLC, glutamate-cysteine ligase; TNR, trinucleotide.
[a]Higher ratio of high-risk genotypes was observed in CLZ responders compared to CLZ nonresponders (corrected-*P* = .042).

there has been only one study reporting the association between symptom severities and GSH levels, as measured by ^{1}H-MRS, in patients with schizophrenia.[44] The authors noted a negative relationship between the Scale for the Assessment of Negative Symptoms (SANS) total score and GSH level in patients with schizophrenia. The relationship between GSH levels and symptom severity remains unclear at present. However, it should be noted that the present study included patients either showing response or nonresponse to antipsychotic treatment. Therefore, there was a lack of patients with moderate symptom severity. Therefore, the relationships between GSH levels and symptom severities still remain unclear. Further studies are needed to examine these relationships using larger samples with various symptom severity measures and across different illness phases.

Thus far, 2 studies investigated the correlations between GSH and Glu in patients with schizophrenia. One study reported a positive correlation between ACC GSH and Glu levels both in patients with schizophrenia and HCs.[43] The other study also reported a positive correlation between these in patients with schizophrenia and HCs who had the GCLC low-risk genotype.[27] Consistent with these findings, we found that HCs showed a positive relationship between them. On the other hand, FLR showed a negative relationship between GSH and Glu levels, which is in contrast to the findings from the aforementioned previous studies. In addition, such different relationships between the groups were not observed in the other neurometabolite, suggesting that the differences were not sporadic or simply related to water. However, it should be noted that the previous studies did not classify patients based on antipsychotic response and, accordingly, could include a mixed sample as compared to our sample which was categorized based on status of treatment resistance. Regarding the findings in this study, there are several possible interpretations. FLR may have aberrant functioning in the GSH synthesizing cycle (ie, γ-glutamyl cycle) or GSH-Glu cycle as a reflection of underlying pathophysiology in comparison with CLZ nonresponders and HCs; through the γ-glutamyl cycle, GSH is synthesized from the precursor amino acids Glu, cysteine, and glycine in the cytosol,[45] and also GSH was reported to serve as a reservoir of neural Glu.[20] Alternatively, the administration of CLZ might be responsible for the differences between groups. We observed a negative relationship between GSH and Glu levels only in FLR, while CLZ-treated participants (both CLZ nonresponders and CLZ responders) showed numerically positive relationships, which is similar to that observed in HCs. According to Lee et al, 4 previous postmortem studies consistently found elevations in expression of a gene coding for a subunit of the GSH synthesis enzyme, glutamate-cysteine ligase modifier (GCLM), in brains of CLZ-treated patients with schizophrenia compared to non-CLZ-treated patients.[46] Thus, it may be possible that CLZ has modulating effects on GSH-Glu neurotransmission by affecting GCLM expression. Still, it remains unclear what mechanism underlies the difference in the correlations between GSH and Glu levels based on antipsychotic treatment response.

Our study demonstrated a higher proportion of high-risk GCLC genotype in CLZ responders compared to

CLZ nonresponders. However, it should be noted that the study also included participants with different ethnicities. First, the classification of high risk/low risk of GCLC genotype was provided based on European Caucasian populations (Swiss and Danish).[26] In addition, the probabilities of variance of GCLC genotypes were significantly different between ethnicities.[47] Further studies are warranted to assess the effects of GCLC genotypes on the risk of schizophrenia and response to antipsychotic treatment, including non-Caucasian ethnicities.

We did not find any effects of GCLC genotypes on GSH levels or on correlations between GSH and glutamatergic neurometabolite levels in the dACC. Our null findings may partially be attributable to the mixed ethnicities of our study as the previous study by Xin et al included only European Caucasian samples. It was only in low-risk genotype samples that the authors found lower ACC GSH levels in patients compared to HCs and a positive correlation between GSH and Glu levels in the ACC.[27] Moreover, participants in the present study consisted of chronically medicated patients, while Xin et al included Caucasian patients in earlier stages of illness (mean = 2.6 years).[27] Furthermore, as previously mentioned, both GSH and Glu levels in the dACC might be affected by antipsychotics including CLZ. Thus, these factors may have led to our null finding regarding a relationship between GCLC genotypes and GSH levels or correlations between GSH and Glu levels in the dACC.

There are several limitations to our study. First, ^{1}H-MRS is unable to differentiate neurotransmitter or vesicular and metabolic pools of neurometabolites. Second, although neurochemical levels were corrected for CSF fraction, relaxation effects were not considered in this study.[48] Third, due to a cross-sectional design of this study, we failed to assess the impact of the long-term illness and accumulated doses of antipsychotics on the neurochemical levels. Fourth, although this study has included 98 participants, each group consisted of a small sample size. Finally, owing to the cross-sectional design of this study, we were not able to determine the causal relationships between GSH levels and antipsychotic treatment. This question may be better answered through studies employing a prospective, longitudinal design. Other limitations are detailed in supplementary discussion.

In conclusion, the main findings of this cross-sectional ^{1}H-MRS study were: (1) there was no identified difference in dACC GSH levels among CLZ nonresponders, CLZ responders, FLR, or HCs; (2) FLR showed a negative relationship between GSH and Glu levels in the dACC, whereas positive associations were found in HCs; and (3) CLZ responders had a higher ratio of high-risk GCLC genotypes compared to CLZ nonresponders. Future studies are warranted to further elucidate neuroimaging correlates of TRS and the mechanisms of action for CLZ.

Supplementary Material

Supplementary data are available at *Schizophrenia Bulletin Open* online.

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Author Contribution

Study concept and design: Y.I., S.N., G.R., P.G., A.G. Acquisition of data: Y.I., S.N., E.P., P.T., A.B., W.M., S.C., V.D., N.S. Analysis and interpretation of data: Y.I., S.N., E.P., P.T., A.B., S.C., V.D., N.S. Drafting of the manuscript: Y.I., S.N., A.G. Statistical analysis: Y.I., S.N.,

A.G. Critical revision of the manuscript for important intellectual content: Y.I., S.N., E.P., P.T., A.B., F.C., J.K., P.S., S.C., G.R., P.G., V.D., N.S., A.G. Obtained funding: S.N., P.G., A.G. Administrative, technical, and material support: W.M. Study supervision: G.R., P.G., A.G.

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